**Amendments to the Drawings**

There are three sheets of drawings in the application; the first sheet is labeled "Fig. 1," and the third sheet is labeled "Fig. 3." The second sheet is clearly intended to be "Fig. 2" but was inadvertently not labeled. Sheet 2 of the Drawings was amended to add the label "Fig. 2." No new matter has been added. Entry is respectfully requested.

Attachment: Replacement Sheet

Annotated Marked-Up Drawings

## REMARKS

**Amendments to the Claims**

Claims 1, 5, 8, 9, 11, 16, 17, 24, 25, 27, 29-31 and 33 have been amended to more clearly define Applicants' invention and to correct obvious typographical or grammatical errors. Claims 2-4, 6 and 28 have been canceled. Support for the amended claims can be found throughout the application as filed, e.g., in original Claims 4, 24, 28-31 and 33 as filed. No new matter has been added. Entry is respectfully requested.

**Election**

The Examiner stated at page 3, lines 7-11, of the Office Action:

> Claim 1 recites "which is symmetrical in three dimensions" and not *three dimensional symmetry*. As such, "2-fold symmetry" taught by Narayana et al. anticipates claim 1 because it is *symmetrical in three dimensions*. There is nothing in claim 1 that requires "a first oligomer assembly" to have a *three dimensional symmetry* (italicized for added emphasis).

Claim 1 has been amended to more clearly define Applicants' invention, and to require that the first oligomer assembly "has a set of rotational symmetry axes extending in three dimensions." This is supported by the application as filed, e.g., in original Claim 4 as filed. As such, "2-fold symmetry" taught by Narayana *et al.* does not anticipate the amended Claim 1.

Because Applicants' invention is not anticipated by Narayana *et al.*, the shared technical feature of the groups is a special technical feature that provides unity of invention, as discussed in the Reply to Restriction Requirement filed December 13, 2007. Because there is a unity of invention, the Examiner's Restriction Requirement is improper.

Applicants note that the Restriction Requirement is not made final in the Office Action mailed from the USPTO on March 31, 2008. Therefore, Applicants provisionally elect again, with traverse, the claims of Group I (Claims 1-25). Applicants reserve the right to file a continuing application or take such other appropriate action as deemed necessary to protect the non-elected inventions. Applicants do not hereby abandon or waive any rights in the non-elected inventions.

**Amendments to the Drawings**

The Examiner stated at page 4, lines 2-4, of the Office Action:

> New corrected drawing in compliance with 37 CFR 1.121(d) is required in this application because Figure 2 is missing. However, the specification on pg. 4, lines 3-5, describes the content of Fig. 2.

There are three sheets of drawings in the application; the first sheet is labeled "Fig. 1," and the third sheet is labeled "Fig. 3." The second sheet is clearly intended to be "Fig. 2" but was inadvertently not labeled. Sheet 2 of the Drawings was amended to add the label "Fig. 2." No new matter has been added. Entry is respectfully requested.

**Objections to the Specification**

The specification was "objected to for inappropriate notation of an Internet address." See page 4, line 10, of the Office Action. The Examiner also stated at page 4, lines 11-15, of the Office Action:

> On page 4 line 19, Internet address is cited in an unacceptable form. ... The examiner suggests the replacement of Internet citations with appropriate references because Internet pages are subjected to frequent changes and deletions and could be different when the public access the Internet page to view the exactly same information.

The specification has been amended to delete the Internet address on page 4, line 19, of the specification. The "Protein Data Bank" reference cited on page 4, lines 15-17, of the specification describes the Internet address deleted from the specification. This is reflected in the amendments to the specification. No new matter has been added. Entry is respectfully requested.

The abstract of the disclosure was "objected to because it contains a sentence that goes on for 266 words, which exceeds the limitation (150 words) as set forth in MPEP § 608.01(b). The abstract also contains legal phraseologies, such as 'means' and 'said,' which should be avoided." See the paragraph bridging pages 4 and 5 of the Office Action.

The abstract has been amended. The abstract, as amended, is shorter and does not contain legal phraseologies, such as "means" and "said."

The Examiner further stated in the paragraph bridging pages 4 and 5 of the Office Action:

> [T]his application fails to comply with the requirements of 37 CFR 1.821 through 1.825; ... To be in compliance, Applicants should identify nucleotide sequences of at least 10 nucleotides and amino acid sequences of at least 4 amino acids in the specification by a proper sequence identifier, i.e., "SEQ ID NO:" (see MPEP 2422.01). ... See particularly pg. 21, last paragraph of the specification containing nucleic acid sequences, and therefore, those sequences should be represented by proper sequence identifier numbers.

A "Sequence Listing" was filed on November 7, 2005 in both paper form and computer readable form. The "Sequence Listing" filed included four nucleic acid sequences, which correspond to the four nucleic acid sequences in the last paragraph of page 21 of the specification, respectively. The specification has been amended to identify the four nucleic acid sequences therein by their respective SEQ ID NOs. No new matter has been added. Entry is respectfully requested.

**<u>Objection to Claims 5, 6 and 13</u>**

Claims 5, 6 and 13 were objected to because of informalities.

The Examiner stated, "the recitation of '[[...]]' is not compliant with the rules as set forth in 37 CFR § 1.121 (c) (2)." See page 6, lines 15-16, of the Office Action.

It appears that the Examiner was referring to Claims 5, 6 and 16, not Claims 5, 6 and 13, because Claim 13 has never been amended.

Claim 6 has been canceled, thereby rendering the objection moot.

Claims 5 and 16 have been amended in the instant Amendment in compliance with the rules as set forth in 37 C.F.R. § 1.121(c)(2). No new matter has been added. Entry is respectfully requested.

**<u>Rejection of Claims 1-25 under 35 U.S.C. § 112, second paragraph</u>**

Claims 1-25 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regard as the invention. See page 7, lines 8-10, of the Office Action.

Claims 2-4 and 6 have been canceled, thereby rendering the rejection moot.

The Examiner stated, "Claim 1-25 are unclear and confusing in the recitation of the word 'respective" in many different phrases." See page 7, lines 11-12, of the Office Action.

Each recitation of "respective" has been removed from Claims 1, 5, 7-17 and 19-25. Claim 18 has one recitation of "respective" in "said further monomers of the protomers fused to respective first monomers." This recitation is not indefinite. Because both the further monomers and the first monomers are in plural form, the recitation of "respective" clearly identifies the first monomer-further monomer pair on an individual basis.

The Examiner also stated, "Claim 1-25 are unclear and confusing in the recitation of the word "first" in many different phrases." See page 9, lines 3-4, of the Office Action.

The recitation of "first" used in the pending claims are relative to the recitation of "further." It is used as a label which distinguishes from, e.g., "further monomer" and "further oligomer assembly." The use of "first" is not indefinite.

In summary, pending Claims 1, 5 and 7-25 are not indefinite under 35 U.S.C. § 112, second paragraph.

**Rejection of Claims 1-25 under 35 U.S.C. § 112, first paragraph**

Claims 1-25 were rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. See page 9, last two lines, of the Office Action.

Claims 2-4 and 6 have been canceled, thereby rendering the rejection moot.

The Examiner stated, "The instant claims are directed to a genus of protein lattices." The Examiner also stated, "The specification discloses only a single representative species of a protein lattice comprising a fusion protein comprising the human ferritin heavy chain (HFH) and the E. coli PurE." The Examiner further stated, "this single disclosed species fails to provide adequate written description for a genus of protein lattices," See page 10, line 4 and page 11, lines 4-6, 15 and 16, of the Office Action.

Even though at the time the instant application was filed, laboratory production of a protein lattice in accordance with the present invention had only been demonstrated for the quoted example of human HFH and *E. coli* PurE, this is sufficient to support the scope of the claims, because of the special nature of the present invention.

The present invention differs from a typical protein invention in that it is not concerned directly with the chemical and biochemical properties of the protein as such. Rather, the present invention is concerned with a principle based on the symmetry of the quaternary structure of the proteins. One of the contributions provided by the present invention is that the internal symmetries of an oligomer assembly can be used to design and build a new class of protein lattices. As the contribution is made at this level of generality, it is appropriate that the scope of the claims is made at this level of generality. In some ways, this particular invention is more clearly analogous to an invention in the field of mechanical engineering, rather than an invention in the field of biotechnology.

The specification contains a very detailed disclosure of the principles of how monomers of oligomer assemblies having particular symmetries can be chosen to produce a protomer which will assemble into a lattice. This is set out as follows.

(a) Page 4, lines 8-19 explains that protein lattices can be designed by selecting oligomers having appropriate symmetries.

(b) Page 4, line 26 through page 8, line 3 describes the principles by which the symmetries of the lattice derives from the symmetry axes of the oligomer assemblies.

(c) Page 10, line 4 through page 16, line 28 describes numerous specific examples of the combinations of symmetries of the oligomer assemblies which allow a protein lattice to form, for example as enumerated in Tables 1 and 2.

(d) As the invention is based on the principle that oligomer assemblies of appropriate symmetries can be used to build a protein lattice, the actual identity of the individual proteins is less important than their symmetry. Page 4, lines 13-19 explains that the symmetries of oligomer assemblies are generally known. Furthermore, Table 3 gives examples of some common proteins of different symmetry groups which are mentioned as the examples in Tables 1 and 2. In other words, protomers having appropriate symmetries can be selected by choosing symmetries of each oligomer assembly from Tables 1 and 2 and then selecting specific proteins having those symmetries from Table 3.

Referencing <u>University of California v. Eli Lilly and Co.</u> and <u>Enzo Biochemical Inc. v. Gen-Probe Inc.</u>, the Examiner stated at the paragraph bridging pages 10-11 of the Office Action:

> To fully describe a genus of genetic material, which is a chemical compound, applicants must (1) fully describe at least one species

> of the claimed genus sufficient to represent said genus whereby a skilled artisan, in view of the prior art, could predict the structure of other species encompassed by the claimed genus and (2) identify the common characteristics of the claimed molecules, e.g., structure, physical and/or chemical characteristics, functional characteristics when coupled with a known or disclosed correlation between function and structure, or a combination of these.

Applicants respectfully submit that both these requirements are clearly met by the instant application. Requirement (1) is met by the parts of the application (a)-(d) set out above which clearly allow the skilled person to predict structures having the required symmetries to form a lattice. Requirement (2) is met because a contribution provided by the present invention is that certain symmetries allow a protein lattice to form. Thus the "common characteristics" that need to be identified in the case of the present invention are the symmetries of the resultant protein lattice, which again are taught by the parts of the application (a)-(d) set out above.

The Examiner also stated that "the specification is silent about what the three dimensional symmetry the fusion protein comprising HFH and PurE is." See page 11, lines 13-14, of the Office Action.

Applicants respectfully disagree with the Examiner. The application discloses the symmetries of human HFH and *E. coli* PurE at page 11, lines 29-32, i.e., that human HFH belong to an octahedral point group and *E. coli* PurE belongs to a dihedral D4 point group. In view of the teaching that assembly causes the rotational symmetry axis to align, the skilled person is clearly taught the cubic symmetry of this lattice, in the paragraph bridging pages 11 and 12 of the Office Action and as also shown in FIG. 1.

The Examiner further stated at page 12, lines 4-9, of the Office Action:

> Without adequate guidance with respect to the genus of protein lattices as claimed, one of skill in the art would not have recognized that the genus of protein lattices, encompassing widely variant species having essentially any structure, can be used in extremely diverse applications as listed in pg. 25, i.e., catalyzing biotransformations, data storage, display, charge separation, nanowire, motor, mould and X-ray crystallography.

Applicants submit that these uses all derive from the symmetry and structure of the protein lattice which is described as set out above. Thus, it is in fact the case that the relevant properties can be recognized. In addition, with the exception of X-ray crystallography, none of

the above mentioned uses are set forth in the instant claims. With respect to X-ray crystallography, the specification has detailed description of how the protein lattice of the present invention can be used in X-ray crystallography, e.g., at page 24, line 7 through page 8, line 1.

In summary, pending Claims 1, 5 and 7-25 meet the written description requirement under 35 U.S.C. § 112, first paragraph.

**<u>Rejection of Claims 1-25 under 35 U.S.C. § 112, first paragraph</u>**

Claims 1-25 were rejected under 35 U.S.C. § 112, first paragraph, "as failing to comply with the enablement requirement, because the specification, while being enabling for a protein lattice comprising a fusion protein comprising the human ferritin heavy chain (HFH) and the E. coli PurE, ..., does not reasonably provide enablement for any protein lattice having a regular structure with any repeating unit repeating in three dimensions." See page 13, first paragraph, of the Office Action.

Applicants respectfully disagree with the Examiner.

As discussed above, the application describes how to design a protomer which has appropriate symmetries to build into a protein lattice. The techniques which then need to be applied to make the protomers and then assemble them are routine in the art.

The form and production of the protomers is described at page 8, line 4 through page 10, line 3 and at page 19, line 2 through page 21, line 8. All of these techniques are routine. Essentially these techniques simply require production of a fusion proteins which was commonplace at the time the instant application was filed. The form and production of the protomers are also taught in WO 00/68248, filed May 8, 2000 and published November 16, 2000, the entire teachings of which are incorporated by reference in the present application. See page 8, lines 4-8, of the application as filed.

Similarly, the assembly process is described on page 21, lines 9-23. Again these techniques are routine. Essentially, it is required only that the protomers are mixed in conditions allowing assembly of the oligomer assemblies (albeit in two stages in the case of heterologous protomers), which was straightforward at the time the instant application was filed. Suitable conditions for the assembly process are also disclosed in WO 00/68248, the entire teachings of

which are incorporated by reference in the present invention. See page 21, lines 13-14, of the application as filed.

It is not alleged that every protomer having the requisite symmetries will form a lattice. However, the present application explains the factors involved in the selection of proteins to maximize the chances of assembly of a lattice, for example, at page 8, line 31 through page 10, line 3.

Applicants attach hereto as Exhibit A, a copy of a document filed at the European Patent Office on August 16, 2006 in connection with European Patent Application No. 03753741.2 which corresponds to the instant U.S. application. The document is an annex to Response to the Communication of February 10, 2006 from the European Patent Office. The document describes an additional experimentally demonstrated 3D regular protein lattice in accordance with the present invention. This example of post-filing success is strong evidence that the instant application is enabling.

The Examiner stated that "the specification is silent about what the three dimensional symmetry of the fusion protein comprising HFH and PurE is." See page 15, lines 8-10, of the Office Action.

As discussed in the previous section, the application discloses the 3D symmetry of the exemplified protomer comprising human HFH and *E. coli* PurE.

The Examiner also stated that the specification is "silent about how the aforementioned protein lattice comprising HFH and PurE can be used in any of the applications listed on pg. 25, i.e., catalyzing biotransformations, data storage, display, charge separation, nanowire, motor, mould and X-ray crystallography." See page 15, lines 10-13, of the Office Action.

As discussed above, these uses all derive from the symmetry and structure of the protein lattice which is described as set out above. In addition, with the exception of X-ray crystallography, none of the above mentioned uses are set forth in the instant claims. With respect to X-ray crystallography, the specification has detailed guidance on how the protein lattice of the present invention can be used in X-ray crystallography, e.g., at page 24, line 7 through page 8, line 1.

The Examiner further stated, particularly with respect to Claim 6, "the specification does not establish: (A) regions of any monomer and protomer which may be modified without

affecting the desired functions of said monomers and protomer, i.e., the ability of fuse or non-covalently interact together to form a protein lattice while having a three-dimensional symmetry; (B) the general tolerance of any monomer and protomer to modification and extent of such tolerance without affecting the aforementioned desired functions; (C) a rational and predictable scheme for modifying any monomer and protomer of any protein lattice with an expectation of obtaining the desired activity/utility." See page 17, first paragraph, of the Office Action.

Claim 6 has been canceled, thereby rendering the rejection moot.

In summary, once the principle with which the invention is concerned (see the previous section for discussions on the principle) has been taught, as in the specification with ample guidance, it is straightforward to conceive of and implement protein lattices comprising protomers other than human HFH and *E. coli* PurE without undue experimentation. Therefore, pending Claims 1, 5 and 7-25 meet the enablement requirement under 35 U.S.C. § 112, first paragraph.

**<u>Rejection of Claims 1-25 under 35 U.S.C. § 102(b)</u>**

Claims 1-25 were rejected under 35 U.S.C. § 102(b) as being anticipated by Padilla et al. (Nanohedra: Using symmetry to design self assembling protein cages, layers, crystals, and filaments, PNAS, 2001, Vol. 98, No. 5, pg. 2217-2221) in view of the evidentiary reference Hestenes (retrievable from the Internet at <http://modelingnts.la.asu.edu/pdf/crystalsymmetry.pdf>). See page 18, lines 14-18, of the Office Action.

Padilla et al. discloses regular protein structures based on symmetrical oligomer assemblies capable of self-assembly. See title and abstract of Padilla et al.

The present invention is concerned with a new technique for building "a protein lattice having a regular structure with a repeating unit repeating in three dimensions," and uses "protein protomers which each comprise at least two monomers fused together, the monomers each being monomers of an oligomer assembly into which the monomers are assembled for assembly of the protomers into the lattice." See instant Claim 1.

Given that different terminology is used in the present application and in Padilla et al., it is useful first to set out the correspondence between the terminology used in the two cases.

Firstly, the term "fusion protein" is used in Padilla et al. in the same sense as the term "protomer" in the present application. See page 1, lines 8-12, of the application as filed, which refers to "fusion protein" in WO 00/68248 as having the same meaning as "protomer" in the instant application. WO 00/68248 and Padilla et al. both have the same teachings. The inventive entity of WO 00/68248 is exactly the same as the list of authors of Padilla et al.

Secondly, the term "oligomerization domain" is used in Padilla et al. in the same sense as the term "monomer" in the present application. See page 1, lines 8-12, of the application as filed, which refers to "oligomerization domains" in WO 00/68248 as having the same meaning as "monomers" in the instant application. Therefore, the description in Padilla et al. of a "fusion protein" comprising "oligomerization domains" fused together has the same meaning as the description in the present application of a "protomer" comprising "monomers" fused together.

Thirdly, the term "structure" as used in Padilla et al. to describe the regular structure into which the "oligomerization domains" assemble has the same meaning as the term "oligomer assembly" as used in the present application to describe the structure or assembly into which the "monomers" assemble.

Fourthly, it is noted that Padilla et al. also uses the term "structure" to define the extended structure into which a large number of fusion proteins assemble. This use of the term "structure" in Padilla et al. must be distinguished from the use of the term "structure" in Padilla et al. to describe the structure or "oligomer assembly" into which oligomerization domains (or "monomers") assemble, that is, in isolation from the fusion protein. This extended "structure" of fusion proteins (or "protomers") described in Padilla et al. refers to a large class of structures including (1) a "protein lattice" as the term is used in the present application to mean regular structure with a repeating unit repeating in three dimensions, and (2) other extended structures, such as a discrete shell or cage or a structure which repeats in one or two dimensions.

In considering the present invention, it is important to distinguish between, on one hand, the geometrical relationship between oligomerization domains (or "monomers") within a fusion protein (or "protomer") and, on the other hand, the geometricl relationship or symmetry between oligomerization domains (or "monomers") within a structure (or "oligomer assembly") into which they assemble.

In both the present invention and Padilla et al., complex structures are built up based on the self-assembly of the monomers (or "oligomerization domains") into an oligomer assembly (or "regular structure"). However, there is an important distinction between the manner in which the symmetry of the lattice is derived from the protomers. This distinction not only means that the lattices of the present invention are different from any lattice disclosed in Padilla et al., but also leads to the lattices of the present invention being easier to assemble.

In Padilla et al., the oligomer assemblies are all dimers or trimers, i.e., having a single rotational symmetry axis of order two or three. The symmetry of the resultant extended structure is dependent on the geometrical relationship between the two oligomer assemblies within the regular structure. The structures are dependent on the choice of particular angles, for example as shown in Fig. 1*b* and set out in Table 1 of Padilla et al. This means that the structure is reliant on careful selection or design of the linking group between the oligomerization domains (or "monomers") within a single fusion protein (or "protomer"). Padilla et al. explains the importance of this in the paragraphs on page 2217, column 1, line 45 through column 2, line 13 and relies on the linking group being an α-helix to achieve the correct angles.

By way of illustration, the effect of the geometric relationship between the oligomerization domains (or "monomers") within a fusion protein (or "protomer") is clearly seen from a comparison of Figs. 1*d* and 1*e* in Padilla et al. In these cases, two different extended regular structures are formed from a fusion protein (or "protomer") consisting of the same two oligomerization domains (or "monomers"), but with different angles between oligomerization domains (or "monomers") within a given fusion protein (or "protomer"). In Fig. 1*d* an angle of 0° causes the fusion protein (or "protomer") to assemble into a crystalline layer repeating in two dimensions, whereas in Fig. 1*e* an angle of 35.1° causes the fusion protein (or "protomer") to assemble into a cage.

In contrast, the present invention uses a different technique from that taught in Padilla et al. to design a protomer (or "fusion protein") which assembles into a protein lattice having a regular structure in three dimensions. In particular, this is achieved by:

(1) the protomer (or "fusion protein") comprising a "first monomer [(or 'oligomerization domain')] of a first oligomer assembly [(or 'regular structure')] which has a set of rotational symmetry axes extending in three dimensions"; and

(2) the protomer (or "fusion protein") further comprising at least a "further monomer [(or 'oligomerization domain')] of a further oligomer assembly [(or 'regular structure')], each further oligomer assembly having a rotational symmetry axis of the same order as one of the set of rotational symmetry axes of the first oligomer assembly and being aligned with the one of the set of rotational symmetry axes of the first oligomer assembly." (see instant Claim 1, as amended)

The result of the symmetries of the first and further oligomer assemblies having rotational symmetry axes of the same order N is that on assembly the oligomer assemblies align with the rotational symmetry axes of the further oligomer assemblies aligned with the rotational symmetry axes of the first oligomer assembly, that is, with an N-fold fusion therebetween. This is for the reasons described at page 6, lines 13-29 of the present application as filed. This is important and a clear distinction from the teachings of Padilla et al.

Firstly, it means that the symmetry of the lattice derives from the arrangement and symmetry of the rotational symmetry axes within the oligomer assembly, that is, from the internal symmetry of the oligomer assemblies. In contrast, in Padilla et al., the symmetry of the extended structure results from the design of the linking groups.

Secondly, it inherently provides an N-fold fusion between the oligomer assemblies. In contrast, in Padilla et al. there is a single-fold fusion between the oligomer assemblies.

Claim 1, as amended, has clear novelty over the teachings of Padilla et al.

Firstly, Padilla et al. does not teach that the protomers each comprise a first monomer which is "a monomer of a first oligomer assembly which has a set of rotational symmetry axes extending in three dimensions" (emphasis added), because the monomers of Padilla et al. are dimers and trimers each having a single rotational symmetry axis, i.e., of order two or three.

In respect of this feature in original Claim 4 (now in amended Claim 1), the Examiner relied on the disclosure in Padilla et al. of a protein cage in Fig. 1*e* having 12 different rotational symmetry axes. See page 20, lines 2-6, of the Office Action. However, Claim 1 as amended requires that the oligomer assembly has a set of rotational symmetry axes extending in three dimensions.

This point of distinction is also clearly pointed out in the specification as filed. When comparing and contrasting the present invention to WO 00/68248, which has the same teachings as Padilla et al., the specification as filed states at page 1, lines 23-30:

> Thus, the lattices suggested in WO-00/68248 having a regular structure repeating in three dimensions are formed from protomers comprising two monomers of respective dimeric or trimeric oligomer assemblies which are symmetrical about <u>a single rotational axis</u>. The relative orientation of the two monomers is selected to provide a specific angle of intersection between the rotational symmetry axis of the two oligomer assemblies. Thus, there is <u>a single fusion</u> between the two oligomer assemblies and the relative orientation of the oligomer assemblies is controlled by careful selection of the linking group providing the fusion. (emphasis added)

Secondly, Padilla et al. does not teach that the protomers comprise at least a further monomer which is a "monomer of a further oligomer assembly, each further oligomer assembly having a rotational symmetry axis of the same order as one of the set of rotational symmetry axes of the first oligomer assembly and being aligned with the one of the set of rotational symmetry axes of the first oligomer assembly."

Consequently, for the lattices of the present invention, the symmetry is derived from the internal symmetry of the oligomer assemblies and there are inherently N-fold fusions between the oligomer assemblies.

Therefore, the protein lattice set forth in amended Claim 1 is novel over the teachings of Padilla et al.

The Examiner asserted that "monomer" is not defined in the specification, and interpreted "monomer" as "a chemical compound that can undergo polymerization" according to the Merriam-Webster dictionary. See page 19, 5-12, of the Office Action.

As discussed above, "monomers" have the same meaning as "oligomerization domains" in WO 00/68248, the entire teachings of which is incorporated by reference in the instant application. See page 1, lines 10-12, and page 8, lines 7-8, of the application as filed. Therefore, the dictionary definition assigned by the Examiner is not appropriate in interpreting "monomers" as used in the instant application.

The Examiner stated at page 20, lines 11-13, of the Office Action, "Claim 7 is anticipated because said monomers or protomers are fused via linking group, i.e., bromoperoxidase which forms trimers, and M1 matrix protein of influenza virus which forms dimers."

It appears that the Examiner interpreted bromoperoxidase and M1 matrix protein of influenza virus taught in Padilla et al. as linking groups. This is not correct. Both bromoperoxidase and M1 matrix protein of influenza virus taught in Padilla et al. are "oligomer assemblies." One monomer of bromoperoxidase and one monomer of M1 matrix protein form a protomer (or "fusion protein") via a linking group, "a nine-residue helical linker." See the paragraph bridging pages 2217 and 2218 of Padilla et al.

For the reasons set forth above, Claim 1, as amended, is not anticipated by Padilla et al. in view of the evidentiary reference Hestenes under 35 U.S.C. § 102(b). Because Claim 1 is not anticipated by Padilla et al. in view of the evidentiary reference Hestenes under 35 U.S.C. § 102(b), and because Claims 5 and 7-25 are all dependent from Claim 1, Claims 5 and 7-25 are not anticipated by Padilla et al. in view of the evidentiary reference Hestenes under 35 U.S.C. § 102(b), either.

**<u>Provisional rejection of Claims 1-25 on the ground of nonstatutory obviousness-type double patenting</u>**

Claims 1-25 were "provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-31 and 33 of copending Application No. 11/807922." See page 22, lines 20-22, of the Office Action.

After the claims are allowed in the instant application, Applicants will address any double patenting rejections which may be caused by the allowance of the claims of the instant application.

**CONCLUSION**

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned.

Respectfully submitted,

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Annex to Response to Communication of 10.2.06

The additional work relevant to protein lattices in accordance with the present invention carried out since submission of the present application is as follows. Herein, protein structures formed by protomers of fused monomers of oligomer assemblies are referred to as "crysalins", this being a term coined by the present inventors.

1) Volumetric crysalins

There has been experimentally demonstrated an additional 3D regular protein lattice in accordance with the present invention and comprising a first oligomer assembly is a small heat shock protein (SHS) having $p_4$ symmetry (octadehral or cubic symmetry) and the further oligomer assembly is the streptavidin/streptag assembly having $d_2$ symmetry (dihedral symmetry of order 2). A model of the assembled protein lattice is shown below (Figure 1A).

Experimentally, an SHS-streptag fusion protein (protomer) has been expressed in E. coli to give a yield of 75 mg.l$^{-1}$ bacterial culture. The fusion protein (protomer) has been purified by streptactin affinity followed by superose 6 size-exclusion chromatography. Upon mixing the SHS-streptag and streptavidin components, the SHS-streptag/streptavidin protein lattice forms. Concentration by desiccation at high temperature yields clear materials (Figure 1B) comparable with those produced by the Ferritin/PurE fusion.

These materials can be completely dissolved by addition of millimolar concentrations of biotin, a competitor of streptag for the streptavidin binding site, confirming that they are crosslinked by the intended interaction. Formation of this protein lattice assembly at extremely low concentrations gives rise to particles small enough to be visualised by negatively stained transmission electron microscopy. This technique reveals a lattice ordered on the molecular scale, conforming with the design (Figure 1C).

A B C

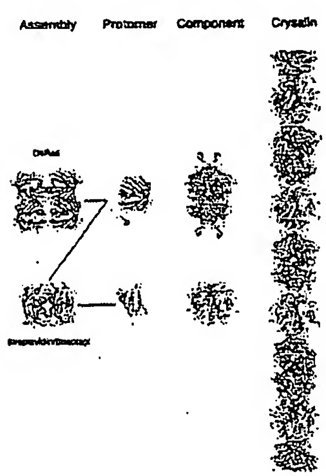
Figure 1. A volumetric crysalin formed using SHS and Streptavidin/StreptagI assemblies.
A Schematic showing the crysalin design.

B Shards of the protein lattice formed after component mixing (typical size ~0.5mM on longest edge).
C Negatively stained EM showing a small segment of this crysalin (N.B. Due to the low contrast at this scan magnification, the lattice is more easily visualised when squinting).

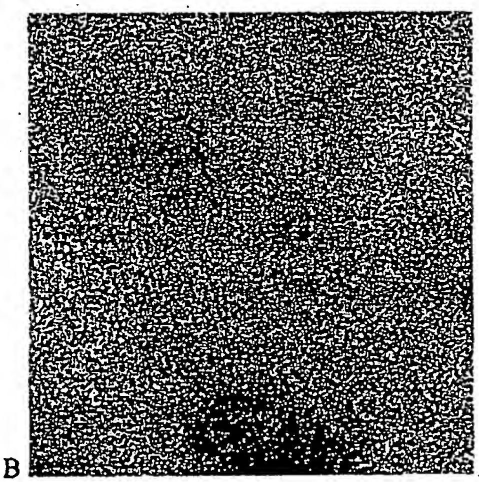
**2) Linear crysalins**

A further generalisation of the crysalin principle has been achieved using DsRed and streptavidin/streptag as the two oligomer assemblies in the protomer, each having $d_2$ symmetry (dihedral symmetry of order 2). The assembly schema for this system and its hypothetical lattice are illustrated below (Figure 2A). Due to the symmetries of the two oligomer assemblies, this produces a protein chain, not a protein lattice which repeats in three dimensions. Whilst not therefore in accordance with the present invention, the principles involved are the same.

The DsRed-streptag fusion protein has been expressed in E. coli to give a yield of 100 mg.$l^{-1}$ bacterial culture. The protein was purified by heating to 65°C for 20 minutes, followed by centrifugation to remove denatured aggregates. The high thermal stability of DsRed means that this protocol provides purification to > 90%. Upon mixing the fusion protein with streptavidin, molecular fibres of alternating DsRed/streptavidin form. These have been visualised using negatively stained transmission electron microscopy (Figure 2B) which shows the production of a structure conforming with the hypothetical structure.




A



B

Figure 2. A linear crysalin formed using DsRed and Streptavidin/StreptagI assemblies.
A Schematic showing the crysalin design.
B Negatively stained EM showing numerous small segments of this linear crysalin. Circular projections of ferritin molecules (included as a reference) are also visible.

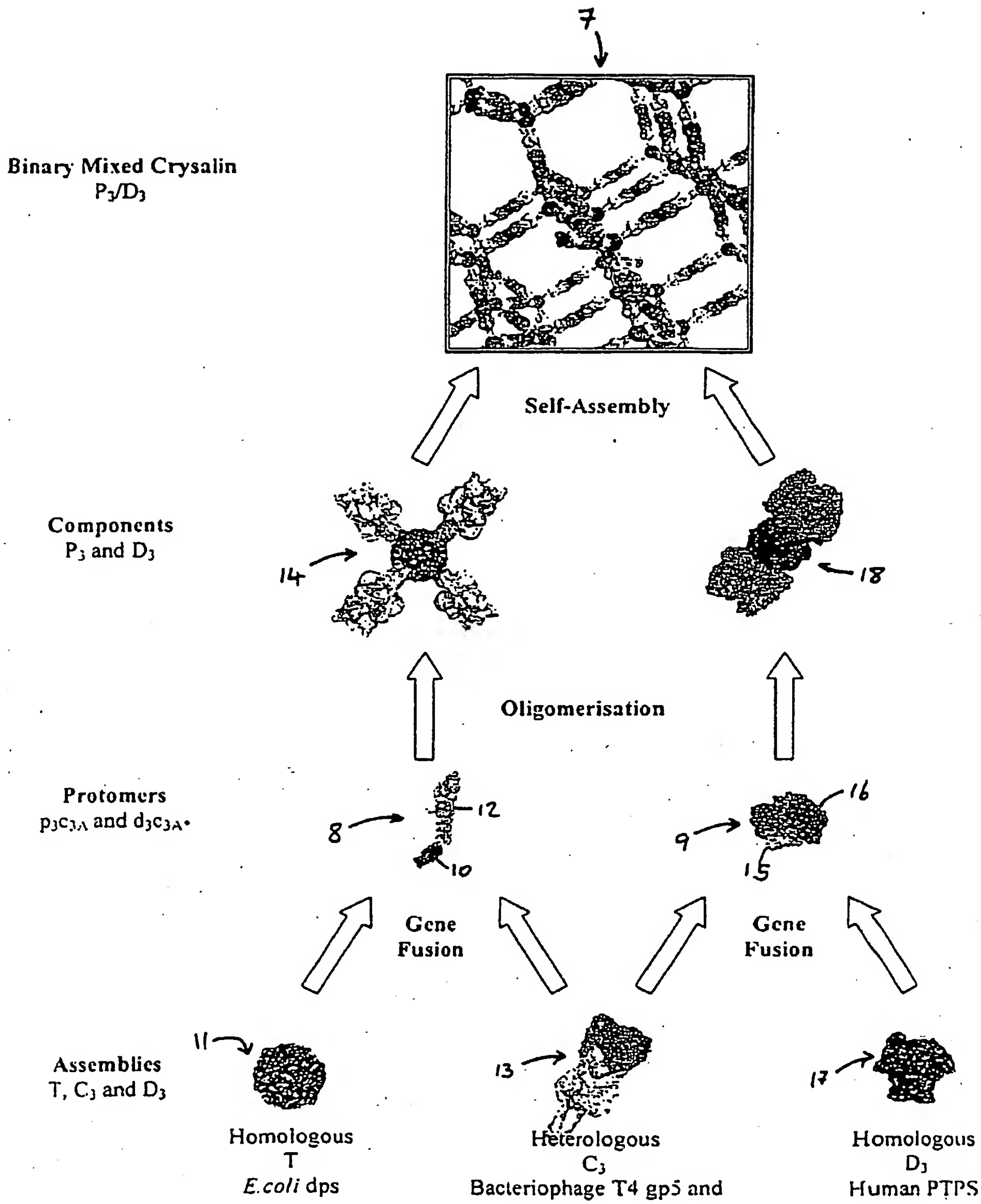

7
Binary Mixed Crysalin
$P_3/D_3$
Self-Assembly
Components
$P_3$ and $D_3$
14
18
Oligomerisation
Protomers
$p_3c_{3A}$ and $d_3c_{3A^*}$
8
12
10
9
16
15
Gene Fusion
Gene Fusion
Assemblies
T, $C_3$ and $D_3$
11
13
17
Homologous
T
*E.coli* dps
Heterologous
$C_3$
Bacteriophage T4 gp5 and
Homologous
$D_3$
Human PTPS

Fig. 2